

Umbilical Cord Blood Transplantation: Clinical Challenges

Shilpa Sharma & Gurudutta Gangenahalli*

Review Article

Division of Stem Cell Gene Therapy Research, Institute of Nuclear Medicine & Allied Sciences (INMAS), Delhi-110054, India.

Abstract

Umbilical cord blood transplantation (UCBT) is becoming a salvage treatment in lieu of bone marrow or peripheral blood stem cell transplantation in patients with hematological disorders who lack human leucocyte antigen (HLA) identical stem cell donor. Additionally, this procedure has demonstrated reduced recurrent acute and chronic graft-versus-host disease, reduced relapse rates, long-term immunological recovery and comparable overall survival when compared with other unrelated allogeneic hematopoietic stem cell transplantation. UCBT is further elucidated by its easy procurement, expeditious availability and more tolerance for HLA mismatches. Discerningly, UCBT is followed by notable delays in the rates and kinetics of neutrophil and platelet engraftment and higher transplant related mortality. This is related in part to the inherently lessened number of hematopoietic stem and progenitor cells in UCB grafts. Several strategies have been assayed to overcome relatively limited number of stem cells, such as multiple UCB transplants and *ex vivo* expansion of HSCs. In this review, we have compiled the current clinical research investigations to provide reader with a brief glimpse into the remarkable potential UCB possess for transplantation.

Keywords: Hematopoietic Stem and Progenitor Cells; Umbilical Cord Blood Transplantation; *ex vivo* Expansion; Engraftment.

*Corresponding Author:

Dr. Gurudutta Gangenahalli PhD,
FRSC (UK), Head, Division of Stem Cell Gene Therapy Research, Institute of Nuclear Medicine & Allied Sciences (INMAS), Delhi-110054, India.
Tel: 91-11-23905144
Fax: 91-11-25737049
Email: gugdutta@rediffmail.com

Received: October 09, 2015

Accepted: December 07, 2015

Published: December 10, 2015

Citation: Shilpa Sharma, Gurudutta Gangenahalli (2015) Umbilical Cord Blood Transplantation: Clinical Challenges. *Int J Stem Cell Res Transplant* 03(6), 134-141. doi: <http://dx.doi.org/10.19070/2328-3548-1500023>

Copyright: Gurudutta Gangenahalli® 2015. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Introduction

Hematopoietic stem cells (HSCs) are the prototype stem cells endowed with the potential of restoring hematopoiesis in patients with hematological malignancies. Clinical transplantation results are best obtained by HLA identical donor or closely related donor. In spite of nearly 13 million registered volunteer donors worldwide, nearly half of the patients do not have a closely matched HLA donor and two-thirds of patients do not get suitable related donor. In such a situation, availability of unrelated donor (URD) from bone marrow (BM), peripheral blood stem cells (PBSC) and umbilical cord blood (UCB) escalate the applicability of HSCT. Though BM and PBSC transplantation have proven record of

success, only 40-50% of unrelated BM and PBSC donor setting provide prompt graft source for patients [1]. In the early 1980s, Broxmeyer et al introduced UCB as a potential source of HSCs and progenitor cells which provided impetus to perform the first UCB transplant for a patient with fanconi anemia in 1988 [2, 3]. The paucity of HSCs in UCB grafts has been a major limitation of UCB transplantation. UCB grafts have been shown to contain ten-fold lower cellular dose (nucleated and CD34⁺ cells) compared to BM and PB transplantation, which has translated into delayed engraftment and immune reconstitution [4]. However, there is a recent report that demonstrates that there are actually more HSCs in UCB than previously realized [5]. A brief exposure to ambient oxygen in native conditions of hypoxia reportedly decreases recovery of long-term repopulating HSCs and increases progenitor cells in UCB. HSC collection in grossly hyperoxic conditions compared to the BM microenvironment [6] compromises the survival of HSCs through the induction of the mitochondrial permeability transition pore (MPTP) and ROS production. The MPTP inhibitor cyclosporin A protects HSCs during collection in air, allowing collection of more number of HSCs in UCB for potential clinical benefit [5].

In unrelated UCB banks, UCB units are rapidly available as “off the shelf” product for narrowing the gap of a growing population of patients who do not have an identified HLA matched relative. Moreover, use of a frozen graft allows scheduling of stem cell collections and date of transplant without losing repopulation capacity on post-thawing [7]. Frozen UCB units can be stored for long duration and then thawed for efficient recovery of HSCs and progenitor cells in relevance to UCB banking and transplantation, Broxmeyer et al. evaluated recovery of functionally-intact hematopoietic progenitor cells for up to 23.5 years in comparison to pre-freeze numbers from the same donor. Highly

efficient recovery (80%-100%) consisting of highly proliferating multipotential hematopoietic progenitors and granulocytes was obtained. CD34⁺ cells isolated from UCB cryopreserved for up to 21 years showed recovery of self-renewing HSCs, differentiated T-lymphocytes and endothelial colony forming cells [8]. Also, these UCB units are tested for infectious diseases prior to storage and are HLA-typed. In comparison with BM or PB graft, UCB have less stringent HLA matching requirements, which allows the use of UCB units with greater mismatch [9]. Permissive HLA mismatching allows searching of a suitable unit for even racial or ethnic minorities. Non-invasive procedure to acquire UCB cells [10]; lack of risk to the donor, and lower risk of acute and chronic graft-versus-host disease (GVHD) in pediatric patients following UCBT are value-added advantages compared to BMT and PBSCT [11]. However, initial hope has been tempered by low immune reconstitution in case of UCB graft compared to other sources such as BM and PBSC. Total nucleated cells (TNCs) and CD34⁺ cells dose in UCB unit are predominant decisive factors for engraftment and survival after UCBT [12-14]. The average TNC dose in a UCB graft is less than ten-fold if compared with average BM graft. Patients who received cell doses less than 1.8×10^7 TNCs and 1.7×10^5 CD34⁺ cells per kilogram of the recipient body weight had inferior engraftment and survival rates [14]. This contributes to increased risk of graft failure, delayed immune reconstitution and higher transplant related mortality [14, 15]. The recovery times for neutrophils (>500 cells/mm³) and platelets (20,000 cells/mm³) were both delayed for UCBT recipients compared to BMT recipients (27 days versus 18 days and 60 days versus 29 days) [9, 16]. The cumulative incidence of non-engraftment after UCBT varies from 10 to 20%. Furthermore, the non-availability of donor lymphocyte infusions prevents application in post-transplant cellular immunotherapy to invigorate donor-derived immunity to treat infections, mixed chimerism, and disease relapse [9].

Single Umbilical cord blood transplantation

Initial reports on the use of UCB as a donor type for hematopoietic cell transplantation were based on the use of single UCB grafts and largely limited to paediatric patients [12, 14, 17-20]. The cohort of the Cord Blood Transplantation (COBLT) prospective study in 191 paediatric patients resulted in 57% overall survival (OS) at 1 year and 77% for very high-risk patients [21]. Results were confirmed with studies from registry and single-centre analysis displaying disease-free survival (DFS) of 50%-60% with early-stage and 10%-30% with advanced-stage of disease, thus demonstrating favorable outcomes in children with malignant and non-malignant hematological diseases. Further, a comparative pediatric study of 99 patients with acute leukemia transplanted with myeloablative UCB graft and 262 MUD BM transplants showed significantly reduced rates of acute and chronic GVHD [20]. However, study resulted in delayed neutrophil (32 versus 18 days) and platelet (81 versus 29 days) recovery times in UCB graft recipients. After promising results in children, the initial UCBT experience with adults was poor [22]. Developments in adult single UCBT followed over the next years with advanced supportive care and higher infused cell dose [23, 24].

Double umbilical cord blood transplantation

Further, it was realized that the salient features for effective engraftment in both pediatric and adult patients in UCBT are the cell dose (TNC or CD34⁺ cells) and HLA-matching of the graft

[25, 26]. Eurocord reported that when a single UCB unit (6/6 or 5/6 HLA-matched) does not contain adequate number of cells (TNC $> 2.5 \times 10^7$ /kg), double UCB unit is recommended, to achieve a combined TNC dose $> 3.0 \times 10^7$ /kg [25]. Barker et al. [27] suggested that if the UCB unit is only 4/6 HLA matched, then the cell dose should be as high as 5.0×10^7 per kg. Even higher doses are recommended if the single UCB unit is only 4/6 HLA-matched. In another study, two UCB units, each of which has no more than 2/6 HLA mismatches (low resolution typing of HLA-A and -B or high resolution typing of HLA-DRB1) were infused into adults and large children [15, 28]. The recipients of 1-antigen-mismatched UCB units with a lower cell dose showed similar engraftment to 2-antigen-mismatched units. 1-antigen-mismatched UCB units with a higher cell dose showed superior engraftment [20]. Study showed that UCB dose is more important factor for engraftment after UCBT than the HLA mismatch. It was further suggested that UCB unit selection need to be done by considering matching at HLA-C for units that are matched at HLA-A, -B, -DRB1 or in the presence of a single locus mismatch at HLA-A, -B or DRB1 [29, 30].

Utilization of double UCBT platform disseminated extensively allowing treatment for large children patients as well as adult patients, applying various preparative regimens and post-transplant immunosuppression [31-34]. Recently, a study showed superior OS, DFS and similar transplant related mortality (TRM) in patients who received double UCB units compared with single UCBT [35]. A lower relapse risk was noticed in patients receiving two CBUs for acute leukemia, possibly through an enhanced graft-versus-leukemia effect [36]. Phase I clinical trials in 23 adult leukemic patients who received double UCB units following myeloablative conditioning, displayed long-term hematopoiesis from single UCB donor in 76% patients at day 21 and in 100% patients by day 100. Initially, both units contribute for engraftment, finally, only one UCB predominates [28]. Although reasons are not yet clearly elucidated, the implication of immune mediated mechanisms has been suggested [37]. CD3⁺ cell dose was the only factor associated with single unit predominance [38]. To ameliorate clinical outcomes after UCBT, a contemporary approach of reduced intensity conditioning (RIC) regimens emerged involving administration of high-dose cyclophosphamide, fludarabine, melphalan, rabbit anti-thymocyte globulin and low-dose total body irradiation before transplantation [39]. Blood and Marrow Transplant Clinical Trial Network conducted two parallel multicentre prospective phase II clinical trials for patients without a suitable related donor. The preparative RIC regimen incorporated cyclophosphamide, fludarabine, and 200 cGy of total body irradiation in both trials, dUCBT (BMT-CTN 0604) and haplo-BMT (BMT-CTN 0603). Although, 1-year cumulative TRM was higher after UCBT and lower in haplo-marrow transplant (24% versus 7%), however, relapse rate was higher after haplo-marrow transplantation (31% versus 45%). 1-year probability of OS and progression-free survival (PFS) recorded was 54% and 46% after dUCBT, whereas 62% and 48% in case of haplo-BMT, respectively. The CI of grade II-IV acute GVHD was 40% after dUCBT and 32% following haplo-BMT at day 100. The CI of neutrophil recovery was 94% after dUCBT and 96% after haplo-BMT at day 56. Haplo-BMT showed much better consequences (less GVHD, TRM and high OS, PFS) compared to dUCBT. Number of patients in BMT-CTN trials were relatively small and non-randomized, so larger follow-ups are needed [40]. Results showed efficacy and safety of study. These phase II trials endorsed the value of double UCBT as an alternative donor source and set the stage for a

multi-center, phase 3 randomized trial with RIC and dUCBT versus HLA haplo-BMT for patients with hematologic malignancies (BMT-CTN 1101).

The major disadvantage of using double cord blood transplants is that it is associated with the increased level of GVHD compared to that of single cord blood transplants [41]. Also, current studies demonstrated no significant variation in the rate of engraftment in patients receiving one or two CBUs [28, 41]. Double UCB units significantly increase cost and demand on cord banks to collect more units. Therefore, it may be best to find a way to get back to single cord blood transplants. A recently reported prospective, multicenter, randomized, phase III comparative study showed similar outcomes in pediatric patients [42]. Results showed that similar overall rate of engraftment (89% versus 86%), chronic GVHD (28% versus 31%), risk of relapse at 1 year (12% versus 14%), or disease-free survival (68% versus 64%) in single versus double UCBT. Even by double UCBT, the number of useful cells obtained were limited and the kinetics of mature blood cells recovery after transfusion were not ideal, so these cells showed little direct therapeutic benefits for period up to 3 weeks. These limitations to stem cell transfusion are tackled by approach of *ex vivo* or *in vivo* expansion of UCB cells before infusion to enhance their quality and usefulness for transplantation.

Ex vivo expansion of UCB cells

UCB cells show high regenerative potential because of longer telomere lengths and high cytotoxicity due to rich density of primitive nature killer cells, therefore, current strategies are focused on the development of technologies to generate greater numbers of hematopoietic stem and progenitor cells (HSPCs) from given fixed cellular yield obtained from an individual UCB unit [43]. There are currently numerous approaches under investigation that include co-infusion of accessory cells with UCB cells, notch mediated expansion, and utilization of small molecular weight compounds to achieve *ex vivo* expansion [44].

Cytokine-mediated expansion

With consistent efforts to achieve cytokine mediated *ex vivo* expansion since a decade, cytokine-mediated expansion methods have been translated to the clinic for autologous and allogeneic uses. As cytokines result in expansion of mature HSCs, it is expected that these committed progenitor cells would enhance absolute neutrophil count recovery. In a study, CD34⁺ cells were isolated from fraction of UCB unit (40-60%) and expanded in liquid culture with cytokines (SCF, G-CSF, TPO, and megakaryocyte growth and differentiation factor, MGDF) *in vitro*. Expanded CD34⁺ cells were co-infused with non-expanded units to 37 patients (median TNC dose of $0.99 \times 10^7/\text{kg}$) following myeloablative conditioning. This approach resulted in 28 days (range, 15-49) for neutrophil recovery and 106 days (range, 38-345) for platelet recovery. Thus, study showed similar duration for hematopoietic recovery compared with non-expanded UCB unit [45]. 40% of patients developed grade III-IV acute GVHD. Study showed feasibility but failed to achieve an improvement in neutrophil and platelet engraftment.

The efficacy of cytokines demonstrated clinical relevant expansion when they were considered in the context of specific cellular response of low oxygen concentration. CD34⁺ cells from UCB were cultured with cytokines SCF, Flt-3 ligand, TPO and G-CSF

in anti-oxidant media for 14 days. TPO stabilize transcription factor 'hypoxia-inducing factor-1 α ' through generation of mitochondrial reactive oxygen species. Study resulted in an extensive amplification of total cells (CD45⁺, CD33⁺, CD19⁺ cells by ~350-fold), committed progenitors (colony-forming cells, ~130-fold) and CD34⁺ cells (~100-fold) [46]. Similar study was performed using MGDF in place of TPO for 12 days. Hypoxic culture resulted in 400-fold hematopoietic expansion of total cells, 80-fold for CD34⁺ stem cells and 150-fold committed progenitors [47]. This protocol is already employed in an ongoing clinical trial (NCT01034449). *Ex vivo* expanded UCB graft by this procedure proved advantageous for long-term hematopoietic reconstitution to generate rapid, complete and sustained donor engraftment in transplanted patients [48]. Recently, a novel small molecule agonist of c-MPL-NR-101, exhibited an even more pronounced effect on the HSPCs than TPO alone [49].

Co-infusion of UCB unit with haploidentical mesenchymal stromal cells

Co-culture with supporting matrix of mesenchymal stromal cells (MSCs) bestows BM stromal microenvironment to promote proliferation and differentiation of hematopoietic cells. MSCs cause immunosuppression as they reduce T-cell proliferation and secrete cytokines [50]. Exact mechanisms remain unknown. Thus, MSCs along with HSCs are considered to serve in lowering GVHD and improve engraftment rates [51]. Using double UCBT platform and a myeloablative conditioning regimen, Shpall and colleagues conducted phase I clinical trial by combining un-manipulated UCB unit (Elizabeth J. Shpall, University of Texas M.D. Anderson Cancer Center, oral communication, November 5, 2010) with a unit that has been expanded in co-culture with cytokines (SCF, Flt-3 ligand, G-CSF, and TPO) and either third-party haploidentical family member MSCs or "off-the-shelf" MSCs (NCT00498316). Co-culture facilitated 40-fold expansion of hematopoietic cells. On day 14, expanded unit was infused following the infusion of the un-manipulated UCB unit. Long-term engraftment was provided by the unexpanded unit in the majority of patients by 11 months after transplantation. Median time of 15 days (range, 9-42) for neutrophil engraftment in 31 (97%) patients and median time of 40 days (range, 13-62) for platelet engraftment in 26 (81%) patients was observed. One patient died before engraftment [52]. Though no enhancement in engraftment was detected, study provided the rationale that UCB could be expanded in MSC co-culture prior to transplantation and set the stage for a randomized trial to compare un-manipulated double UCB transplantation in which one of the units is expanded in MSC co-cultures. In another study examining MSCs potential, eight patients received haploidentical MSCs (median dose $2.1 \times 10^6/\text{kg}$ in addition to UCB (median TNC $3.1 \times 10^7/\text{kg}$) on the day of UCB transplant and three patients received additional dose of MSCs on day 21. There were no harmful side effects related to infusion of MSCs. All patients achieved neutrophil recovery at a median of 19 days (range, 9-28 days) and six patients achieved platelet engraftment at a median of 53 days (range, 36-98 days). With a median follow-up of 6.8 years, 5 patients remained alive and disease free. Rates of engraftment, GVHD and survival were reportedly comparable to historic groups with no serious adverse effects with MSC infusion [53]. In a pilot study, nine patients received myeloablative conditioning followed by UCB transplants with co-infusion of MSCs and T-depleted HSC from a third-party donor. All patients achieved neutrophil engraftment at a median of 12 days with full CB chimerism at a median of 51

days. The maximum cumulative incidence of platelet engraftment was 88% at a median of 32 days. However, no significant differences in UCB engraftment were observed if compared to a control group of 46 transplants from the same center not receiving MSCs. Four patients in the MSC group developed grade II acute GVHD. Two patients who developed steroid-refractory GVHD were given repeated infusions of MSCs and both showed complete remission. Last two achieved complete remission after therapeutic infusions of MSCs [54]. In a similar study, 13 pediatric patients co-transplanted with UCB and parental MSCs, showed less grade III-IV GVHD and no significant disparity in engraftment and grade II-IV acute GVHD compared to 39 matched historic controls [55]. De Lima et al. [56] reported the results of 31 patients receiving two CBUs, one of which was co-cultured with MSCs *ex vivo*. The median TNC dose infused was $8.34 \times 10^7/\text{kg}$. This *ex vivo* culture system expanded TNC and CD34⁺ cells by a median factor of 12.2 and 30.1, respectively. Of the 24 patients who received *ex vivo* expanded cells, 23 achieved neutrophil engraftment, at a median time of 15 days (range, 9-42 days), and 18 had sustained platelet engraftment, at a median time of 42 days (range, 15-62 days). Although the expanded unit improves early hematopoietic recovery, it is the un-manipulated unit that usually provides long-term engraftment at 6 months [56]. These studies illustrated safety and efficacy in the presence of third-party MSCs and confirm a potential role for UCB derived HSPC expansion methodologies.

Low molecular weight synthetic compounds

Another alternative strategy for the augmentation of the hematopoietic homing or proliferation potential is the brief treatment of the double UCB graft to small molecule modulators targeting to enhance hematopoietic engraftment. Direct addition of small synthetic compounds to the culture medium is safe and easy approach in the light of *ex vivo* expansion protocols.

Tetraethylenepentamine (TEPA): Out of several LMW agonists/antagonists screened and assessed for stem cell expansion; the most encouraging results were obtained in phase I trial with copper chelator, TEPA. It is based on the inherent property of copper deficiency to block hematopoietic maturation without affecting development of progenitor cells [57]. Administration of copper chelator, TEPA, in UCB derived CD34⁺ CD38⁻ cells along with stimulatory cytokines (SCF, Flt-3 ligand, TPO and IL-6) promote CD34⁺ cells expansion by 17-fold at three weeks and 159-fold at seven weeks compared to input cell numbers [58]. TEPA mediated *ex vivo* expanded HSPCs showed improvement in NOD/SCID engraftment at eight weeks following transplantation [59, 60]. In a phase I/II clinical trial, single CD133⁺ cells were isolated from segregated portion of UCB unit and expanded with early acting cytokines (SCF, Flt-3 ligand, TPO, IL-6 and TEPA) for 21 days. The median TNC fold expansion was 219 (range, 2-260). The expanded unit was infused 24 hrs after infusion of un-manipulated portion in 10 patients after myeloablative conditioning. Despite the infusion of a relatively low number of total nucleated cells per kilogram (median of $1.8 \times 10^7/\text{kg}$), 9 of the 10 patients engrafted with a median time to neutrophil and platelet engraftment of 30 days (range, 16-46) and 48 days (range, 35-105) respectively after transplantation. There was no increase in non-relapse mortality or the rate of acute GVHD in comparison with historical controls. This approach showed no convalescence in the time to neutrophil or platelet engraftment compared with previous reports [61]. To further evaluate this approach, Gamida

Cell performed prospective multicenter myeloablative UCBT trial (StemEx[®]) in 100 patients with advanced hematological malignancies. Patients were transplanted with a single UCB unit of which CD133⁺ cells from an aliquot of the UCB unit (20-50%) and cultured for 21 days with hematopoietic cytokines and TEPA and transplanted along with a minimum of 10^7 TNCs/ kg from the un-manipulated portion of the same unit (NCT00469729). This study demonstrated the advantage of StemEx[®] over dUCBT historical controls, measured by a significant improvement in day 100 survival (84% versus 72%) and faster early engraftment of neutrophils (21 versus 28 days) and platelets (54 versus 105 days). StemEx yielded a median of 14-fold increase in the number of CD34⁺ cells infused, in comparison to the number of CD34⁺ cells the patients could have received from the entire un-manipulated CBU. Importantly, the CD34⁺ cell dose from the StemEx expanded fraction was associated with time to neutrophil and platelet engraftment and inversely associated with grade III/IV infections during 100 day post-transplant. Out of 101 patients, 16 patients died in the first 100 days. This technical knowledge influences the venture of increasing the number of UCBT being performed while potentially diminishing its short term morbidity and mortality [62].

Prostaglandin E2: A conceivable modulator, the stable prostaglandin E2 (PGE2) derivative 16, 16-dimethyl PGE2 (dmPGE2), was previously identified in a chemical screen using zebra fish embryos to be a potent regulator of vertebrate HSC homeostasis. Mechanistically, dmPGE2 activates G-protein coupled prostaglandin receptors (PTGER2 and PTGER4) and consequently upregulate the expression of genes involved in homing (eg, CXCR4), proliferation (eg, cyclinD1), self-renewal (Wnt signaling) and cell survival (eg, surviving) [63-65]. A recent study revealed PGE2 induce expression of NR4A1 in long-term reconstituting hematopoietic stem cells which is closely associated with HSC quiescence and myeloid-biased HSCs [66]. Studies demonstrated the ability of dmPGE2 to enhance hematopoietic engraftment of HSCs [65, 67]. A phase I clinical trial evaluated the safety and efficacy using treatment with dmPGE2 *ex vivo* in 12 patients following reduced-intensity double UCBT. One of two UCB units was incubated with a stable derivative of PGE2 for two hours. The expanded unit dominated in 9 out of 11 evaluated patients. Results from this study demonstrated safety with accelerated neutrophil recovery (17.5 versus 21 days) of the dmPGE2-treated UCB unit in 10 of 12 treated participants [68]. No instances of primary or secondary graft failure were observed [58]. A phase II study is currently under way [69].

Stemregenin: Aryl hydrocarbon receptor antagonist, stemregenin (SR1) was previously identified by microarray screening of self-renewal regulators to be a potent enhancer of human hematopoietic CD34⁺ cell numbers. Pre-clinical studies showed that 3 week culture of HSCs with SR1 led to a 50-fold increase in CD34⁺ cells and a 17-fold increase in hematopoietic cells that retain the ability to engraft immunodeficient mice compared with uncultured cells or cells cultured with cytokines alone [70]. Aryl hydrocarbon receptors express on HSCs and are implicated in hematopoiesis regulating pathways such as HES-1, Pu.1, C/EBP-beta, β -catenin and others. The precise mechanism whereby aryl hydrocarbon receptor inhibitor might induce HSPC self-renewal remains unknown. SR1 and Notch ligand delta1 synergistically enhance the number of HSPCs [71]. Study showed that UCB cells cultured in combination with SR1, Delta1 and cytokines (IL-6, TPO, SCF, and Flt-3 ligand) for 14 to 16 days, lead to increase

in progenitors by 25-fold and decrease in differentiation to more mature myeloid cells. Furthermore, SR1 alone improved B-cell repopulation better than Delta1 or the combination of Delta1 and SR1 in culture of UCB cells [71]. This study showed economically feasible results to further develop cellular therapy to mitigate the risks associated with delayed myeloid recovery following UCB transplantation.

Nicotinamide: Study revealed that nicotinamide, a SIRT1 inhibitor, in co-culture with SCF, TPO, IL-6 and Flt3 ligand, facilitates expansion of primitive progenitor cells (CD34⁺ CD38⁻ and CD34⁺ CD38⁻ Lin⁻) and enhanced myeloid and T-cell engraftment in murine models [72]. Encouraging results obtained in preclinical studies led to phase I/II clinical trials. A pilot study in 11 adult patients with haematological malignancy was transplanted with myeloablative double UCB unit (NCT01221857). CD133⁻ cells were selected from one UCB unit for expansion and cryopreserved until transplant day, and were co-infused with the expanded UCB. CD133⁻ fraction was co-infused to render original graft's immunocompetent T cells and NK cells to facilitate engraftment of the UCB. Uncultured CD133⁻ cells in combination with an enhanced HSPC dose following *ex vivo* expansion allows NiCord product for long-term engraftment. Study showed 486-fold expansion in neutrophils and 72-fold expansion in numbers of CD34⁺ cells. The median time to engraftment was 13 days, with one patient developing graft failure. NiCord expansion also allowed choosing a smaller but better HLA-matched UCB unit for transplantation. This has the potential to alleviate the frequency and severity of GVHD and improve survival [30]. Extended follow-up with larger number of patients is needed to confirm the safety and durability of clinical cell product (Gamida-cell, Israel).

Notch mediated expansion of UCB

In phase I trial, patients were given myeloablative conditioning, followed by infusion of one un-manipulated UCB unit (2.4x10⁵ CD34⁺ cells/kg) and second UCB unit that has undergone 164-fold *ex vivo* expansion (6x10⁶ CD34⁺ cells/kg) in the presence of immobilized Notch ligand and cytokines (SCF, Flt-3 ligand, TPO, IL-3 and IL-6) [7]. Median time to neutrophil recovery observed was 16 days (range, 7-34) in those receiving the expanded unit which was reportedly faster compared with 26 days (range, 23-26 days) in a concurrent cohort of 20 patients receiving dUCBT with the same conditioning. Study demonstrated aggrandizement in neutrophil engraftment. Engraftment of donor CD33⁺ and CD14⁺ cells predominated initially from the expanded UCB unit but disappeared in a few weeks in favor of non-expanded UCB. Only half of the patients demonstrated predominant engraftment with the expanded UCB unit while another half showed engraftment from the un-manipulated unit, implying that the expanded unit may have facilitated engraftment by the un-manipulated unit. No case of engraftment failure was reported [7]. OS and GVHD risk were tantamount to those receiving un-manipulated UCB grafts. Phase II and III trials are required to further evaluate parameters of occurrence of infection, survival and duration of hospital stay in recipients of UCB grafts.

Solutions to Clinical Challenges

Notwithstanding many encouraging clinical studies for upgrading engraftment rate following UCBT, challenges continue to exist. Delayed immune reconstitution following high-dose chemotherapy and radiation for hematopoietic transplantation is a serious

impediment to progress of UCBT. Developing contemporary approach is to deplete selectively alloreactive T cells from the haploidentical BM donor graft and host (to inhibit both GVHD and graft rejection) before transplantation. Another critical issue for achieving accelerated engraftment is the expansion of the long-term repopulating and mature HSPCs. A more likely advantage of expansion is the ability to use smaller cord blood units, which could further increase the availability of allografts. Notch-mediated expansion of UCB progenitors led to the production of large number of short-term repopulating myeloid progenitor cells. However, analysis is tedious as involve infusion of a second immunocompetent unit, resulting implausible long-term survival of the expanded cells. Exposure of the graft to stemregenin showed considerable progress for expansion in early clinical trials. Combination treatment of donor UCB graft by either co-culture with MSCs, immobilized notch ligand or copper chelation followed by infusion of these cells into conditioned recipients that are previously treated with stemregenin may be a better approach. It is important to consider cost-effective technologies to will affect the application of stem cell expansion methodologies such as using closed and culture system of bioreactors and 3-dimensional lattices which allow effective utilization of media and cytokines [73]. Nanofiber scaffold with different spacers based *ex vivo* expansion strategy for HSCs, while preserving their stem cell characteristics are undergoing progressive research [74]. Effective manipulation of these events may provide UCBT more efficient and efficacious procedure. Still, expansion strategies are in multicenter trials, but none is as yet an established therapy.

Although hematopoietic cell expansion in UCB has shown improvements in engraftment but specific modification or modulation of homing and engraftment may serve as a possible direct approach to make the limited number of available HSPCs more efficient in homing and engraftment. It was examined that CD34⁺ cells from UCB show relatively weak affinity for the BM microvasculature as they contain reduced levels of fucosylation of E- and P-selectin ligands. In a clinical study (#NCT01471067), enforced fucosylation of hematopoietic cells of smaller of the two UCB units, resulted in accelerated engraftment (17 days for neutrophils and 35 days for platelets). Though, later chimerism studies showed that unfucosylated unit dominated in about half the transplants [75]. Larger randomized studies will be required to confirm results in a larger data set and explore the mechanism of enhancement of engraftment. To evade the cell loss before homing, the concept of direct micro-injection of CD34⁺ UCB cells into the BM environment emerged. In Phase I/II trial, this technology was implemented in 32 patients with acute leukemia. All engrafted patients showed full donor chimerism [76]. Median time to neutrophil and platelet engraftment was 23 days (range 14-44) and 36 days (range 16-64) respectively. Study established the safety and efficacy of this procedure. Other experimental studies include inhibition of dipeptidyl peptidase 4 (DPP4, expressed as CD26 on the cell surface) using small peptides or by deletion of CD26, and the upregulation of CXCR4 expression. DPP4 cleaves the chemokine stromal cell-derived factor-1 (SDF-1), and truncated SDF-1 antagonizes signals from full-length SDF-1 α . DPP4 inhibition enhance hematopoietic progenitor cell survival, *ex vivo* cytokine expansion, replating frequency, marrow homing and engraftment capability of limiting numbers of long-term repopulating mouse HSCs into lethally irradiated mice [77]. Inhibition of DPP4 also mediate SDF-1 independent effects as it enhances *in vitro* granulocyte-macrophage progenitor cell proliferation and also enhances recovery of hematopoiesis after stress *in vivo* [78].

Farag et al. evaluated the feasibility and potential efficacy of systemic inhibition of DPP-4 using sitagliptin to enhance engraftment of single unit UCB grafts in adults with hematological malignancies (clinical trial NCT00862719). Clinical trial participants yielded favorable engraftment kinetics of 21 days, shorter time for engraftment than with single UCB transplantation reported in previous studies and showed cumulative incidence of engraftment of 94% at 50 days [79]. On the basis of data from this clinical trial, a semi-mechanistic model based approach was developed to understand sitagliptin concentration and DPP4 activity after single unit UCB transplantation. Simulations showed that twice or three times daily dosage schedules were better than a once daily schedule for maximal DPP4 inhibition at the lowest sitagliptin exposure. This study provides the first pharmacokinetic and pharmacodynamic analysis of sitagliptin and provides a valuable tool for optimization of dose regimens and schedule to enhance engraftment after single UCB transplantation in adult patients [80].

Concluding Remarks

UCB graft is rapidly growing as significant HSC source for adults and remains an important mainstay for treatment in children by showing superior results. We have focused in this review on the results of the various clinical trials to improve engraftment and the approaches to realize expansion strategies. Efforts focused on addressing cell dose limitations using *ex vivo* expansion and increased homing to assess safety and effectiveness are required to continue the advancement in the field of UCBT. Exciting HSC expansion data with use of direct addition of extrinsic modulators may be applicable to clinical settings in the near future. A potential combination of enhancement of proliferation (using stem-regenin, TEPA) and homing (eg PGE2, enhanced fucosylation, intrabone infusion, inhibition of CD26, CXCR4 up-regulation) procedures may result in greater improvement in engraftment capacity than any one procedure itself. Homing procedures may be relatively inexpensive to perform and more widely used without extensive *ex vivo* maneuvers or experience. Haematologists are trying to expand HSCs by exploring gene transfer strategies to manipulate HSCs for amplification. Future efforts to expand HSPCs to enhance the engrafting capabilities of UCB cells will likely make use of more in depth information on intracellular signaling molecules and their networks involved in hematopoietic proliferation, survival, differentiation, homing and migration. Future studies may also develop alternative sources of HSPCs, in particular embryonic stem cells or induced pluripotent stem cells, which may be amenable to many of the expansion methods described herein. During the next few years, we may presume that expedited progress of the encouraging procedures delineated herein will result in appreciably amplified production of HSPCs for clinical implementation.

Acknowledgments

We are thankful to Dr R.P.Tripathi, Director, Institute of Nuclear Medicine and Allied Sciences (INMAS), Brig. S. K. Mazumdar Marg, Delhi-110054 for providing us necessary facilities and support. Authors also thank the Ministry of Defence, Department of Defence Research and Development for research grant. We thank Mrs Namita Kalra for help with flow cytometry, Dr Sudhir Chandna for use of real-time PCR machine. Shilpa Sharma in particular thanks Indian Council of Medical Research (ICMR) for the award of Senior Research Associateship.

References

- [1]. Gluckman E (2009) Ten years of cord blood transplantation: from bench to bedside Br J Haematol 147(2): 192-199.
- [2]. Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, et al. (1989) Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical cord blood from an HLA-identical sibling. N Engl J Med 321(17): 1174-1178.
- [3]. Broxmeyer HE, Douglas GW, Hangoc G, Cooper S, Bard J, et al. (1989) Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. Proc Natl Acad Sci USA 86(10): 3828-3832.
- [4]. Sharma S, Gurudutta GU, Satija NK, Pati S, Afrin F, et al. (2006) Stem cell c-KIT and HOXB4 genes: critical roles and mechanisms in self-renewal, proliferation, and differentiation. Stem Cells Dev 15(6): 755-778.
- [5]. Mantel CR, O'Leary HA, Chitteti BR, Huang X, Cooper S, et al. (2015) Enhancing hematopoietic stem cell transplantation efficacy by mitigating oxygen shock. Cell 161(7): 1553-1565.
- [6]. Sharma S, Gurudutta GU (2014) Adult Hematopoietic Stem Cells: Niche Cross-Talks to Affect the Cell Fate. Niche (in Press) doi: 10.5152/niche.2015.215
- [7]. Delaney C, Ratajczak MZ, Laughlin MJ (2010) Strategies to enhance umbilical cord blood stem cell engraftment in adult patients. Expert Rev Hematol 3(3): 273-283.
- [8]. Broxmeyer HE, Lee MR, Hangoc G, Cooper S, Prasain N, et al. (2011) Hematopoietic stem/progenitor cells, generation of induced pluripotent stem cells, and isolation of endothelial progenitors from 21- to 23.5-year cryopreserved cord blood. Blood 117(18): 4773-4777.
- [9]. Danby R, Rocha V (2014) Improving engraftment and immune reconstitution in umbilical cord blood transplantation. Front Immunol 5: 68.
- [10]. Cohen Y, Nagler A (2004) Umbilical cord blood transplantation--how, when and for whom? Blood Rev 18(3): 167-179.
- [11]. Grewal SS, Barker JN, Davies SM, Wagner JE (2003) Unrelated donor hematopoietic cell transplantation: marrow or umbilical cord blood? Blood 101(11): 4233-4244.
- [12]. Gluckman E, Rocha V (2004) Factors associated with outcome after cord blood transplantation in children with acute leukemia. Eurocord-Cord Blood Transplant Group. Cord blood transplantation for children with acute leukemia: a Eurocord registry analysis. Blood Cells, Molecules & Diseases 33(3): 271-273.
- [13]. Rubinstein P, Carrier C, Scaradavou A, Kurtzberg J, Adamson J, et al. (1998) Outcomes among 562 recipients of placental-blood transplants from unrelated donors. N Engl J Med 339(22): 1565-1577.
- [14]. Wagner JE, Barker JN, DeFor TE, Baker KS, Blazar BR, et al. (2002) Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood 100(5): 1611-1618.
- [15]. Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, McGlave PB, et al. (2005) Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood 105(3): 1343-1347.
- [16]. Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, et al. (2004) Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med 351(22): 2265-2275.
- [17]. Barker JN, Weisdorf DJ, Wagner JE (2001) Creation of a double chimera after the transplantation of umbilical-cord blood from two partially matched unrelated donors. N Engl J Med 344(24): 1870-1871.
- [18]. Locatelli F, Perotti C, Torretta L, Maccario R, Montagna D, et al. (1999) Mobilization and selection of peripheral blood hematopoietic progenitors in children with systemic sclerosis. Haematologica 84(9): 839-843.
- [19]. Michel G, Rocha V, Chevret S, Arcese W, Chan KW, et al. (2003) Unrelated cord blood transplantation for childhood acute myeloid leukemia: a Eurocord Group analysis. Blood 102(13): 4290-4297.
- [20]. Rocha V, Cornish J, Sievers EL, Filipovich A, Locatelli F, et al. (2001) Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. Blood 97(10): 2962-2971.
- [21]. Kurtzberg J, Prasad VK, Carter SL, Wagner JE, Baxter-Lowe LA, et al. (2008) Results of the Cord Blood Transplantation Study (COBLT): clinical outcomes of unrelated donor umbilical cord blood transplantation in pediatric patients with hematologic malignancies. Blood 112(10): 4318-4327.
- [22]. Laughlin MJ, Barker J, Bambach B, Koc ON, Rizzieri DA, et al. (2001) Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. N Engl J Med 344(24): 1815-1822.
- [23]. Robin M, Sanz GF, Ionescu I, Rio B, Sirvent A, et al. (2011) Unrelated cord blood transplantation in adults with myelodysplasia or secondary acute myeloblastic leukemia: a survey on behalf of Eurocord and CLWP of EBMT. Leukemia 25(1): 75-81.
- [24]. Sanz J, Boluda JC, Martin C, Gonzalez M, Ferrá C, et al. (2012) Single-unit

- umbilical cord blood transplantation from unrelated donors in patients with hematological malignancy using busulfan, thiopeta, fludarabine and ATG as myeloablative conditioning regimen. *Bone Marrow Transplant* 47(10): 1287-1293.
- [25]. Rocha V, Gluckman E (2009) Improving outcomes of cord blood transplantation: HLA matching, cell dose and other graft- and transplantation-related factors. *Br J Haematol* 147(2): 262-274.
 - [26]. Rocha V, Labopin M, Sanz G, Arce W, Schwerdtfeger R, et al. (2004) Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med* 351(22): 2276-2285.
 - [27]. Barker JN, Scaradavou A, Stevens CE (2010) Combined effect of total nucleated cell dose and HLA match on transplantation outcome in 1061 cord blood recipients with hematologic malignancies. *Blood* 115(9): 1843-1849.
 - [28]. Brunstein CG, Setubal DC, Wagner JE (2007) Expanding the role of umbilical cord blood transplantation. *Br J Haematol* 137(1): 20-35.
 - [29]. Eapen M, Klein JP, Ruggeri A, Spellman S, Lee SJ, et al. (2014) Impact of allele-level HLA matching on outcomes after myeloablative single unit umbilical cord blood transplantation for hematologic malignancy. *Blood* 123(1): 133-140.
 - [30]. Eapen M, Klein JP, Sanz GF, Spellman S, Ruggeri A, et al. (2011) Effect of donor-recipient HLA matching at HLA A, B, C, and DRB1 on outcomes after umbilical cord blood transplantation for leukemia and myelodysplastic syndrome: a retrospective analysis. *Lancet Oncol* 12(13): 1214-1221.
 - [31]. Bradstock K, Hertzberg M, Kerridge I, Svennilson J, George B, et al. (2009) Single versus double unrelated umbilical cord blood units for allogeneic transplantation in adults with advanced haematologic malignancies: a retrospective comparison of outcomes. *Intern Med J* 39(11): 744-751.
 - [32]. Horwitz ME, Morris A, Gasparetto C, Sullivan K, Long G, et al. (2008) Myeloablative intravenous busulfan/fludarabine conditioning does not facilitate reliable engraftment of dual umbilical cord blood grafts in adult recipients. *Biol Blood Marrow Transplant* 14(5): 591-594.
 - [33]. Kanda J, Rizzieri DA, Gasparetto C, Long GD, Chute JP, et al. (2011) Adult dual umbilical cord blood transplantation using myeloablative total body irradiation (1350 cGy) and fludarabine conditioning. *Biol Blood Marrow Transplant* 17(6): 867-874.
 - [34]. Kai S, Wake A, Okada M, Kurata M, Atsuta Y, et al. (2013) Double-unit cord blood transplantation after myeloablative conditioning for patients with hematologic malignancies: a multicenter phase II study in Japan. *Biol Blood Marrow Transplant* 19(5): 812-819.
 - [35]. Labopin M, Ruggeri A, Gorin NC, Gluckman E, Blaise D, et al. (2014) Cost-effectiveness and clinical outcomes of double versus single cord blood transplantation in adults with acute leukemia in France. *Haematologica* 99(3): 535-540.
 - [36]. Verneris MR, Brunstein CG, Barker J, MacMillan ML, DeFor T, et al. (2009) Relapse risk after umbilical cord blood transplantation: enhanced graft-versus-leukemia effect in recipients of 2 units. *Blood* 114(19): 4293-4299.
 - [37]. Sideri A, Neokleous N, Brunet De La Grange P, Guerton B, Le Bousse-Kerdilès MC, et al. (2011) An overview of the progress on double umbilical cord blood transplantation. *Haematologica* 96(8): 1213-1220.
 - [38]. Ramirez P, Wagner JE, DeFor TE, Blazar BR, Verneris MR, et al. (2012) Factors predicting single-unit predominance after double umbilical cord blood transplantation. *Bone Marrow Transplant* 47(6): 799-803.
 - [39]. Di Bartolomeo P, Santarone S, De Angelis G, Picardi A, Cudillo L, et al. (2013) Haploidentical, unmanipulated, G-CSF-primed bone marrow transplantation for patients with high-risk hematologic malignancies. *Blood* 121(5): 849-857.
 - [40]. Brunstein CG, Fuchs EJ, Carter SL, Karanes C, Costa LJ, et al. (2011) Alternative donor transplantation after reduced intensity conditioning: results of parallel phase 2 trials using partially HLA-mismatched related bone marrow or unrelated double umbilical cord blood grafts. *Blood* 118(2): 282-288.
 - [41]. Brunstein CG, Gutman JA, Weisdorf DJ, Woolfrey AE, DeFor TE, et al. (2010) Allogeneic hematopoietic cell transplantation for hematologic malignancy: relative risks and benefits of double umbilical cord blood. *Blood* 116(22): 4693-4699.
 - [42]. Wagner JE Jr, Eapen M, Carter S, Wang Y, Schultz KR, et al. (2014) One-unit versus two-unit cord-blood transplantation for hematologic cancers. *N Engl J Med* 371: 1685-1694.
 - [43]. Ballen KK, Gluckman E, Broxmeyer HE (2013) Umbilical cord blood transplantation: the first 25 years and beyond. *Blood* 122(4): 491-498.
 - [44]. Munoz J, Shah N, Rezvani K, Hosing C, Bollard CM, et al. (2014) Concise review: umbilical cord blood transplantation: past, present, and future. *Stem Cells Transl Med* 3(12): 1435-1443.
 - [45]. Shpall EJ, Quinones R, Giller R, Zeng C, Baron AE, et al. (2002) Transplantation of ex vivo expanded cord blood. *Biol Blood Marrow Transplant* 8(7): 368-376.
 - [46]. Ivanovic Z, Duchez P, Chevalere J, Vlaski M, Lafarge X, et al. (2011) Clinical-scale cultures of cord blood CD34(+) cells to amplify committed progenitors and maintain stem cell activity. *Cell Transplant* 20(9): 1453-1463.
 - [47]. Duchez P, Chevalere J, Vlaski M, Dazey B, Milpied N, et al. (2012) Definitive setup of clinical scale procedure for ex vivo expansion of cord blood hematopoietic cells for transplantation. *Cell Transplant* 21(11): 2517-2521.
 - [48]. Milpied N, Dazey B, Ivanovic Z, Duchez P, Vigouroux S, et al. (2011) Rapid and sustained engraftment of a single allogeneic ex-vivo expanded cord blood unit (CBU) after reduced intensity conditioning (RIC) in adults: preliminary results of a prospective trial. *Blood (ASH Annual Meeting Abstracts)* 118 Abstract 486.
 - [49]. Nishino T, Miyaji K, Ishiwata N, Arai K, Yui M, et al. (2009) Ex vivo expansion of human hematopoietic stem cells by a small-molecule agonist of c-MPL. *Exp Hematol* 37(11): 1364-1377.
 - [50]. Sato K, Ozaki K, Oh I, Meguro A, Hatanaka K, et al. (2007) Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. *Blood* 109(1): 228-234.
 - [51]. Ringden O, Uzunel M, Rasmussen I, Remberger M, Sundberg B, et al. (2006) Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation* 81(10): 1390-1397.
 - [52]. De Lima M, Robinson S, McMannis J, et al. (2010) Mesenchymal stem cell (MSC) based cord blood (CB) expansion (Exp) leads to rapid engraftment of platelets and neutrophils [abstract]. *Blood* 116 Abstract 362.
 - [53]. Macmillan ML, Blazar BR, DeFor TE, Wagner JE (2009) Transplantation of ex-vivo culture-expanded parental hematopoietic mesenchymal stem cells to promote engraftment in pediatric recipients of unrelated donor umbilical cord blood: results of a phase I-II clinical trial. *Bone Marrow Transplant* 43(6): 447-454.
 - [54]. Gonzalo-Daganzo R, Regidor C, Martín-Donaire T, Rico MA, Bautista G, et al. (2009) Results of a pilot study on the use of third-party donor mesenchymal stromal cells in cord blood transplantation in adults. *Cytotherapy* 11(3): 278-288.
 - [55]. Bernardo ME, Ball LM, Cometa AM, Roelofs H, Zecca M, et al. (2011) Co-infusion of ex vivo-expanded, parental MSCs prevents life-threatening acute GVHD, but does not reduce the risk of graft failure in pediatric patients undergoing allogeneic umbilical cord blood transplantation. *Bone Marrow Transplant* 46(2): 200-207.
 - [56]. de Lima M, McNiece I, Robinson SN, Munsell M, Eapen M, et al. (2012) Cord-blood engraftment with ex vivo mesenchymal-cell coculture. *N Engl J Med* 367(24): 2305-2315.
 - [57]. Peled T, Landau E, Prus E, Treves AJ, Nagler A, et al. (2002) Cellular copper content modulates differentiation and self-renewal in cultures of cord blood-derived CD34+ cells. *Br J Haematol* 116(3): 655-661.
 - [58]. Peled T, Landau E, Mandel J, Glukhman E, Goudsmid NR, et al. (2004) Linear polyamine copper chelator tetraethylenepentamine augments long-term ex vivo expansion of cord-blood derived CD34+ cells and increases their engraftment potential in NOD/SCID mice. *Exp Hematol* 32(6): 547-555.
 - [59]. Peled T, Mandel J, Goudsmid RN, Landor C, Hasson N, et al. (2004) Pre-clinical development of cord blood derived progenitor cell graft expanded ex vivo with cytokines and the polyamine copper chelator tetraethylenepentamine. *Cytotherapy* 6(4): 344-355.
 - [60]. Peled T, Glukhman E, Hasson N, Adi S, Assor H, et al. (2005) Chelatable cellular copper modulates differentiation and self-renewal of cord blood-derived hematopoietic progenitor cells. *Exp Hematol* 33(10): 1092-1100.
 - [61]. de Lima M, McMannis J, Gee A, Komanduri K, Couriel D, et al. (2008) Transplantation of ex vivo expanded cord blood cells using the copper chelator tetraethylenepentamine: a phase I/II clinical trial. *Bone Marrow Transplant* 41(9): 771-778.
 - [62]. Stiff PJ, Montesinos P, Peled T, Landau E, Rosenheimer N, et al. (2013) StemEx® (Copper Chelation Based) Ex Vivo Expanded Umbilical Cord Blood Stem Cell Transplantation (UCBT) Accelerates Engraftment and Improves 100 Day Survival In Myeloablated Patients Compared To a Registry Cohort Undergoing Double Unit UCBT: Results Of A Multicenter Study Of 101 Patients With Hematologic Malignancies. *ASH 2013 Annual Meeting Abstract* 295.
 - [63]. Goessling W, Allen RS, Guan X, Jin P, Uchida N, et al. (2011) Prostaglandin E2 enhances human cord blood stem cell xenotransplants and shows long-term safety in preclinical nonhuman primate transplant models. *Cell Stem Cell* 8(4): 445-458.
 - [64]. Hoggatt J, Singh P, Sampath J, Pelus LM (2009) Prostaglandin E2 enhances hematopoietic stem cell homing, survival, and proliferation. *Blood* 113(22): 5444-5455.
 - [65]. North TE, Goessling W, Walkley CR, Lengerke C, Kopani KR, et al. (2007) Prostaglandin E2 regulates vertebrate hematopoietic stem cell homeostasis. *Nature* 447(7147): 1007-1011.
 - [66]. Land RH, Rayne AK, Vanderbeck AN, Barlowe TS, Manjunath S, et al. (2015) The orphan nuclear receptor NR4A1 specifies a distinct subpopulation of quiescent myeloid-biased long-term HSCs. *Stem cells* 33(1): 278-288.

- [67]. Goessling W, North TE, Loewer S, Lord AM, Lee S, et al. (2009) Genetic interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration. *Cell* 136(6): 1136-1147.
- [68]. Cutler C, Multani P, Robbins D, Kim HT, Le T, et al. (2013) Prostaglandin-modulated umbilical cord blood hematopoietic stem cell transplantation. *Blood* 122(17): 3074-3081.
- [69]. Davies SM (2015) Making a little go a long way. *Blood* 125(19): 2877-2878.
- [70]. Boitano AE, Wang J, Romeo R, Bouchez LC, Parker AE, et al. (2010) Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science* 329(5997): 1345-1348.
- [71]. Dahlberg A, Brashem-Stein C, Delaney C, Bernstein ID (2014) Enhanced generation of cord blood hematopoietic stem and progenitor cells by culture with StemRegenin1 and Delta1(Ext-IgG). *Leukemia* 28(10): 2097-2101.
- [72]. Peled T, Shoham H, Aschengrau D, Yackoubov D, Frei G et al. (2012) Nicotinamide, a SIRT1 inhibitor, inhibits differentiation and facilitates expansion of hematopoietic progenitor cells with enhanced bone marrow homing and engraftment. *Exp Hematol* 40(4): 342-355.e1.
- [73]. Astori G, Adami V, Mambrini G, Bigi L, Cilli M, et al. (2005) Evaluation of ex vivo expansion and engraftment in NOD-SCID mice of umbilical cord blood CD34+ cells using the DIDEKO "Pluricell System". *Bone Marrow Transplant* 35(11): 1101-1106.
- [74]. Chua KN, Chai C, Lee PC, Ramakrishna S, Leong KW, et al. (2007) Functional nanofiber scaffolds with different spacers modulate adhesion and expansion of cryopreserved umbilical cord blood hematopoietic stem/progenitor cells. *Exp Hematol* 35(5): 771-781.
- [75]. Popat U, Mehta RS, Rezvani K, Fox P, Kondo K, et al. (2015) Enforced fucosylation of cord blood hematopoietic cells accelerates neutrophil and platelet engraftment after transplantation. *Blood* 125(19): 2885-2892.
- [76]. Frassoni F, Gualandi F, Podestà M, Raiola AM, Ibatucci A, et al. (2008) Direct intrabone transplant of unrelated cord-blood cells in acute leukaemia: a phase I/II study. *Lancet Oncol* 9(9): 831-839.
- [77]. Christopherson KW, Hangoc G, Mantel CR, Broxmeyer HE(2004) Modulation of hematopoietic stem cell homing and engraftment by CD26. *Science* 305(5686): 1000-1003.
- [78]. Broxmeyer HE, Hoggatt J, O'Leary HA, Mantel C, Chitteti BR et al. (2012) Dipeptidylpeptidase 4 negatively regulates colony-stimulating factor activity and stress hematopoiesis. *Nat Med* 18(12): 1786-1796.
- [79]. Farag SS, Srivastava S, Messina-Graham S, Schwartz J, Robertson MJ, et al. (2013) In vivo DPP-4 inhibition to enhance engraftment of single-unit cord blood transplants in adults with hematological malignancies. *Stem Cells Dev* 22(7): 1007-1015.
- [80]. Velez de Mendizabal N, Strother RM, Farag SS, Broxmeyer HE, Messina-Graham S, et al. (2014) Modelling the sitagliptin effect on dipeptidyl peptidase-4 activity in adults with haematological malignancies after umbilical cord blood haematopoietic cell transplantation. *Clin Pharmacokinet* 53(3): 247-259.